Synopsis for Registration as Research Scholar

for

Doctoral Degree

on

Characterizing Abnormality in Pathological Images Using AI Techniques

By

Kharat Pravin Ramrao

Research Scholar

Supervisor

Dr. Abhijeet V. Nandedkar

Department of Electronics and Telecommunication Engineering

Shri Guru Gobind Singhji Institute of Engineering and Technology,

Nanded-431606 (M.S.) India.

Characterizing Abnormality in Pathological Images Using AI techniques

Abstract:

While high-resolution pathology images lend themselves well to 'data hungry' deep learning algorithms, obtaining exhaustive annotations on these images for learning is a major challenge. Here will see some self-supervised conventional neural network (CNN) framework to leverage unlabeled data for learning generalizable and domain invariant representations in pathology images.

Data processing and learning has become a spearhead for the advancement of medicine, with pathology and laboratory medicine has no exception. The incorporation of scientific research through clinical informatics, including genomics, proteomics, bioinformatics, and biostatistics, into clinical practice unlocks innovative approaches for patient care. Computational pathology is burgeoning subspecialty in pathology that promises a better-integrated solution to whole-slide images, multi-omics data, and clinical informatics. However, computational pathology faces several challenges, including the ability to integrate raw data from different sources, limitation of hardware processing capacity, and a lack of specific training programs, as well as issues on ethics and larger societal acceptable practices that are still solidifying. The establishment of the entire industry of computational pathology requires far-reaching changes of the three essential elements connecting patients and doctors: the local laboratory, the scan center, and the central cloud hub/portal for data processing and retrieval. Computational pathology, unlocked through information integration and advanced digital communication networks, has the potential to improve clinical workflow efficiency, diagnostic quality, and ultimately create personalized diagnosis and treatment plans for patients. This review describes clinical perspectives and discusses the statistical methods, clinical applications, potential obstacles, and future directions of computational pathology.

1. Introduction:

A tumor's micro environment (TME) is characterized by the presence and spatial organization of different types of cells in and around a tumor. To assess the role of the TME milieu in tumor initiation, development, invasion, and outcome, it is important to accurately identify different types of cells. For example, while the *epithelial cells* are important because many cancers originate there, the tumor infiltrating *lymphocytes*(TILs) are associated with the outcomes of various cancers [1], [2]. Similarly, the tumor associated *macrophages* (TAMs) and *neutrophils* also influence diverse processes such as angiogenesis, neoplastic cell mitogenesis, antigen presentation, matrix degradation, and cytotoxicity in various tumors [3].

Although hematoxylin and eosin (H&E) stained tissue slides are often used for the primary diagnosis due to their low cost, they are not very good for differentiating nuclei of different types of cells. For instance, epithelial nuclei within the tumor vary in their appearance across organs, and these can appear small and dense, just like lymphocytes, within a necrotic tissue. Macrophages can be mistaken for the other cells that they may have just started digesting. The multi-lobular structure of neutrophils can be missed depending on their angle of rotation.

More importantly, the manual identification of individual cells takes substantial time and is prone to inter- and intra-observer variability. Thus, automated detection of nuclei of different types of cells using artificial intelligence (AI) can reduce time, costs, and errors to set up for the analysis of the TME.

To build generalization and robust algorithms for segmenting and recognizing nuclei of various types of cells, the computer vision community needs a large, diverse, curated, and annotated dataset. Previously, several public datasets have been released for nucleus segmentation, but the annotated nuclei were either not further classified into different types [4]–[8], or not sourced from multiple organs [8], or not curated to the level of individual nuclei [9]. We, therefore, prepared the multi-organ nucleus segmentation and classification (MoNuSAC) dataset with over 46,000 hand-annotated nuclei covering 71 patients, 31 hospitals, four organs, and four cell types. Our focus was on identifying epithelial and three types of immune cells that are important for characterizing the TME. We sourced the raw images from The Cancer Genome Atlas (TCGA) repository [10]. Based on the dataset, the computer vision community was invited to participate in the MoNuSAC2020 challenge that featured as a satellite event in the International Symposium on Biomedical Imaging (ISBI) 2020. Thirteen teams from 11 countries successfully completed the challenge. Post challenge submissions were opened for another month after the challenge workshop due to a popular demand and the COVID-19 situation, which attracted four additional submissions and two re-submissions. Computational credits for the top ten teams to refine their techniques and a GPU prize for the challenge winner were sponsored by the Nvidia Corporation.

After the challenge, we confirmed that the top few techniques compared favorably with inter-human agreement for the challenge metric. We also checked that ensembling the top few techniques led to only a modest improvement in accuracy. Thus, we hope that the challenge dataset and competing algorithms will be used in the near future to characterize the TME for cancer prognostication and immune response assessment. With this paper, we have released the details of the competing techniques and MoNuSAC training and testing dataset to the public under the creative commons license [11]. We revisit other data sources and techniques for nucleus segmentation and classification. We describe the preparation of MoNuSAC2020 training and testing datasets, and the choice of evaluation metric. We summarize the techniques developed by the challenge participants and their results, post-challenge experiments , and the conclusions.

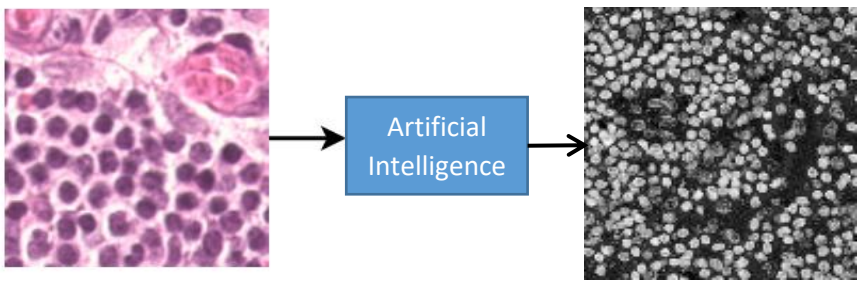

Fig1: Artificial Intelligence applications on Lymph nodes images

2. Literature Review:

Earlier nucleus segmentation algorithms largely used non machine learning approaches, such as watershed segmentation, morphological operations, color-based thresholding, and variants of active contours [7], [12]–[15]. However, these methods failed to generalize across diverse nuclear morphological present in multiple organs and tissue types. Furthermore, these algorithms did not perform well for segmenting touching and overlapping nuclei [5]. Due to these reasons several machine learning algorithms were developed for accurate and Generalization nucleus segmentation. Earlier machine learning approaches focused on the extraction of hand-crafted features based on color, intensity, and texture from H&E stained tissue images to distinguish between nuclear and non-nuclear pixels using traditional algorithms such as random forests, support vector machines, etc. [16]–[18]. However, feature selection for such algorithms relied on domain knowledge and such algorithms also used trial-and-error for improving nucleus

Segmentation performance. Additionally, these methods treated simultaneous nucleus segmentation and classification as two distinct decoupled tasks. More detailed reviews of these nucleus segmentation algorithms appear in [4], [5], [19], [20].

The advent of large annotated datasets and supervised deep learning models overcame the limitations of aforementioned hand-crafted feature based approaches. As algorithms based on deep learning that scale performance more effectively to the availability of labeled data started outperforming prior techniques, the focus of computer vision research shifted from engineering of features to that of neural network architectures and loss functions. For nucleus segmentation, there has been a recent interest in deep neural net architectures inspired from the fully constitutional neural networks (FCN) [5], [8], [21], [22]. These architectures involve the use of encoder-decoder blocks that could systematically transfer features from multiple scales and levels for efficient image segmentation. The U-Net model, a variant of FCN architecture, has given promising results for nucleus and other segmentation tasks [23]. The U-Net model has additional skip connections compared to the FCN for spatially refined semantic segmentation, when touching objects of the same kind need not be recognized as separate entities. Some modifications to the basic U-Net architectures, such as U-Net++ [24], utilize enhanced encoderdecoder architectures and loss functions to improve the *instance segmentation* of individual (possibly crowded) nuclei.

More robust architectures have also been proposed such as W-Net, which uses a two step procedure for contour detection and segmentation map generation for improved nucleus segmentation [25]. Some of the other techniques that make use of the nuclear contours for effective instance segmentation are based on a unified multi-level FCN framework with contour map generation for separating overlapping nuclei instances [18], and a contour-seed pairs learning for robust nucleus segmentation [13]. Another set of popular techniques adopt mask-

RCNNs [11] from the general vision literature for robust instance level nucleus segmentation [23]–[24]. These methods make use of an additional region proposal step to identify potential nucleus locations and following it up with a segmentation step. For nucleus classification, usually a patch centered around a nucleus is input to a neural network, which makes detection or segmentation a prerequisite task. For example, SC-CNN uses a novel neighboring ensemble predictor to label individual nuclei that follows detection by a spatially constrained convolutional neural network [25], while Hover-Net performs simultaneous nucleus segmentation and classification using horizontal and vertical distance maps to resolve and separate clustered nuclei [17]. Recently, mask-RCNN has also been used for the joint segmentation and classification of various types on nuclei in H&E stained tissue images of lung Adenocarcinoma [19]. An exhaustive list of other deep learning based nucleus segmentation and classification techniques can be found in [18].

3. Methodology to be adopted:

We want to make a brief illustration of our hyper spectral pathology image. Each hyper spectral pathology image is composed of line dimension, sample dimension, and wavelength dimension, and comes from our homemade MHSI system which is designed to incorporate with microscopic system for pathology smear analysis, and ultimately for histopathology diagnosis. During image acquisition, one way of light enters the color CCD to take ordinary microscope image; the other way enters the AOTF filter, and then imaged on the grayscale CCD to collect hyper spectral image. The captured image data contained 60 bands, in the spectral range of 550-1000 nm with 7.5 nm spectral resolution. Each band contains 1024×1024 pixels×12 bit/pixel stored in BSQ format, with the spatial resolution of 3μm under 20X magnification and 6μm under 10X magnification.

Each pixel of the 3D data cube presents an N-dimensional spectrum which can be viewed as the representation of spectrum feature for the specific substance that the pixel belongs to. The red curve shows a representation of transmittance spectrum feature of melanoma in a hyperspectral pathology image. When it comes to skin histopathology analysis, it requires comparison among different tissues such as melanoma, vessels or blood cells. Their spectra combined with spatial feature, are believed to make various tissues highly distinguishable, since those features represent the homogeneity within the same tissues and heterogeneity among different tissues. In our datasets, patients were separated into 3 parts to make sure the relevant clinical features matched: 85 for training, 20 for validation and 20 for testing. Then, we used the MHSI system to scan each patient's biopsy specimen to capture 3 to 4 images of size 1024 × 1024 × 60. To accommodate special needs of pathologists for clinical application scenarios, we collected the raw dataset at the magnification of both 10X and 20X.

Given some constrains in data acquisition and implementation, a series of pre-processing methods was applied and discussed in the following sections. Since the whole 3D data were too large to fit into the model, overlapping subsets of 256 × 256 × 16 cubes were obtained from the pre-processed hyper spectral pathology images. Finally, 4760 patches of data cubes were used for training, 1116 for validation and 1120 for testing, among each dataset the distribution of magnification (62% 20X and 38% 10X) remained the same. Based on the outputs of the

proposed 3D fully conventional network (Hyper-net), we illustrated a binary (normal-verse-melanoma) segmentation map and each class of the segmentation map is in company with a probability map.

As mentioned, there are some constrains in data acquisition and implementation such as the emission spectra of the illumination sources, the transmission of the optics in the microscope and the detection sensitivity of the charge coupled device (CCD) camera, resulting in redundant and noisy data to some extent. We followed the band selection strategy in [50] using mutual information as the indicator. The Principle Component Analysis (PCA) was first used to select the most informative component as the reference band. The where $n \in [1, 60]$ represents the band, P() represents the probability distributions of gray scale of the nth band or the reference component R. Moreover, a calibration process is needed beforehand, aimed to eliminate noisy data and obtain the significant characteristic spectra of histopathology tissue. In the spectral imaging microscope under the transmitted illumination system, especially for biomedical applications, the typical method is to use the blank hyper spectral image as the reference image which is acquired at the predetermined wavelengths by imaging a covers lipped slide containing no section. In MHSI system, the blank image is used to characterize the electronic instrument noise. The calibration is calculated by equation (2), where R, B and D represents the raw image, the blank image and the dark image, respectively assessment of mutual information between each band.

4. Importance of study/ Society application:

Most pathology applications of AI are in relatively early development. There are only a few FDA-approved devices that use AI, and these support cervical cytology screening and blood/fluid cell classification. In research settings, machine learning has been used successfully to classify and grade lung cancer, predict prognosis in lung and brain cancer, classify colorectal polyps, diagnose and classify lymphoma, measure breast tumor proliferation, identify metastases in lymph nodes, predict bladder cancer recurrence, diagnose and grade prostate cancer, and identify tumor stroma. A detailed review of machine learning applications in pathology is available. Some of this work has been done as part of machine learning competitions, such as the Camyleon 2016 Challenge and others that compare the performance of algorithms from many research groups. Future applications may calculate scores such as Ki-67 automatically, identify tumor area, count items on slides (tumor-infiltrating lymphocytes, mitoses, organisms, inclusions), predict cancer genetics and prognosis from histologic sections, identify regions of greatest interest on a slide, and triage cases based on slide content. Machine learning has been applied to a number of clinical laboratory tasks, including prediction from laboratory results of cardiac risk, hepatic disease and anemia, endocrine diagnoses, and other conditions. Machine learning has also been used to predict test results based on existing results of other tests, potentially reducing the need for testing, and to autovalidate gas chromatography–mass spectrometry analyses. In aggregate these studies in both anatomic pathology and laboratory medicine show promising results, although most are limited to highly focused tasks using high-quality images and data sets that may not fully generalize to routine operational settings.

A number of startup and established companies are attempting to translate the pathology imaging results into commercial products. It is important to recognize that the research prototypes mentioned above each implement a relatively narrow task within the full set of tasks that a pathologist would carry out to evaluate a tissue specimen. Useful products will need broader capabilities, likely through expansion of the initial limited prototypes to complementary aggregates of individual machine learning algorithms working in concert. These algorithm aggregates must be validated against larger and more varied image sets than have been used thus far. At this early stage of development, the optimal design of machine learning tools for pathology and their fit into the pathology workflow has not been defined, and substantial work remains to be done. Studies in breast cancer metastasis detection suggest that machine learning tools may effectively augment pathologist performance in a workflow that remains pathologist-centric. Studies in radiology, which as a domain is somewhat ahead of pathology in its engagement with machine learning, have reached similar conclusions and results in both domains are compatible with the AMA's proposal that machine learning should support and extend physician performance.

Machine learning may yield tools useful for pathology decision support and education. An augmented reality microscope using machine learning that can highlight and label important features in the field of vision in real time could increase pathologist efficiency and also be useful in residency training or continuing medical education. Similarity search across histopathologic image libraries could suggest differential diagnoses, display groups of similar cases, or identify difficult-to-distinguish images for training purposes. Tools that integrate imaging, genomics, biomarkers, and prognosis prediction could support more comprehensive and useful pathology reports and provide a broader view of disease.

A recent survey of 487 pathologists in 59 countries (22% in the United States) showed that about 80% (380 of 473) expected AI tools to be incorporated into the laboratory in the near future. Although there was some concern about job loss, more than 70% of pathologists (347 of 476) expressed interest in or excitement about the potential new capabilities.Pathologists felt that diagnostic decision-making should remain a predominantly human task but that AI tools could improve the prognostic capability of pathologists while decreasing diagnostic error and improving practice quality and efficiency. These sentiments are generally consistent with the goals for AI recommended by the AMA. Pathologists believed that there were technical challenges in the implementation of AI tools, including methods for validation and performance monitoring, error detection, and a requirement for a fully digital pathology workflow, and that there needs to be better definition of medicolegal responsibility for the output of AI systems. They also felt there was a need for training in the effective use of AI, including strategies for AI system deployment and evaluation of system performance, a sentiment shared with radiologists.

Machine learning will have an impact on pathology and health care because it is able to address problems that are difficult to automate in other ways, and evidence suggests performance will be at least adequate. It is difficult to predict how transformative or disruptive this impact will be until tools based on machine learning that are appropriate for clinical application begin to take shape. In the meantime, it is prudent to become familiar with the principles upon which machine learning is based and the likely performance characteristics of machine learning systems.

Pathologists will use and manage these systems in the future, and in the shorter term they will collaborate with data scientists and engineers to create systems for testing. This review introduces machine learning concepts, terminology, algorithm examples, and development strategies, describes general strengths and weaknesses of machine learning systems, and discusses issues likely to be important in the validation, deployment, and verification of clinical machine learning tools. We intend the review to be for pathologists without a substantial informatics or engineering background, and our goals are to provide an intuitive understanding of key issues in machine learning, improve the ability to collaborate with machine learning experts and read pathology literature about machine learning, and open a gateway to further study.

5. Objective

To provide a background on machine learning for practicing pathologists, including an overview of algorithms, model development, and performance evaluation; to examine the current status of machine learning in pathology and consider possible roles and requirements for pathologists in local deployment and management of machine learning systems; and to highlight existing challenges and gaps in deployment methodology and regulation.

6. Proposed Work Plan / Formulation and Structure of Study:

Year	Proposed Work Plan
2022-23	• Literature survey of image description and related work at international and national level • Study of machine learning, deep learning and AI related subject • Collection of Database • Learn python and other open source programming language • Implement base methods and verify the results • Overcome the lacuna in existing method by investigating new approach • Implement the new approach
2023-24	• Innovate the advancement in proposed approach to get better result. • Analyze and compare the proposed and existing outcomes. • Research Publication • Study the latest techniques to tackle the problem • Design a new approach with latest technique • Analyze and compare the result • Research Publication
2024-25	• Propose the advancement in architecture • Analyze and compare the result • Research Publication • Thesis Writing

References:

1. W. H. Fridman, L. Zitvogel, C. Saut'es-Fridman, and G. Kroemer, "The immune contexture in cancer prognosis and treatment," *Nature Reviews Clinical Oncology*, v14, no. 12, pp. 717–734, 2017.
2. J. J. Havel, D. Chowell, and T. A. Chan, "The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy," *Nature Reviews Cancer*, vol. 19, no. 3, pp. 133–150, 2019.
3. B. Ruffell and L. M. Coussens, "Macrophages and therapeutic resistance in cancer," *Cancer cell*, vol. 27, no. 4, pp. 462–472, 2015.
4. N. Kumar, R. Verma, S. Sharma, S. Bhargava, A. Vahadane, and A. Sethi, "A dataset and a technique for generalized nuclear segmentation for computational pathology," *IEEE transactions on medical imaging*, vol. 36, no. 7, pp. 1550–1560, 2017.
5. N. Kumar, R. Verma, D. Anand, *et al.*, "A multi-organ nucleus segmentation challenge," *IEEE transactions on medical imaging*, vol. 39, no. 5, pp. 1380–1391, 2019.
6. H. Irshad, L. Montaser-Kouhsari, G. Waltz, O. Bucur, J. Nowak, F. Dong, N. W. Knoblauch, and A. H. Beck, "Crowdsourcing image annotation for nucleus detection and segmentation in computational pathology: evaluating experts, automated methods, and the crowd." in *Pacifific Symposium on Biocomputing*, 2015, pp. 294–305
7. S. Wienert, D. Heim, K. Saeger, A. Stenzinger, M. Beil, P. Hufnagl, M. Dietel, C. Denkert, and F. Klauschen, "Detection and segmentation of cell nuclei in virtual microscopy images: a minimum-model approach," *Scientifific reports*, vol. 2, p. 503, Nov. 2012.
8. Naylor, M. La´e, F. Reyal, and T. Walter, "Segmentation of nuclei in histopathology imagesby deep regression of the distance map," *IEEE Transactions on Medical Imaging*,vol.38, no.2ɹ., pp. 448–459, Feb 2019.
9. Gamper, N. A. Koohbanani, S. Graham, M. Jahanifar, S. A. Khurram, A. Azam, K. Hewitt, and N. Rajpoot, "Pannuke dataset extension, insights and baselines," *arXiv preprint arXiv:2003.10778*, 2020.
10. "The cancer genome atlas (tcga)," http://cancergenome.nih.gov/.
11. "Multi-organ nuclei segmentation and classifification challenge (MoNuSAC) 2020[online]," https://monusac-2020.grand-challenge. org/, accessed 11 Aug. 2020.

12. X. Yang, H. Li, and X. Zhou, "Nuclei segmentation using markercontrolled watershed, tracking using mean-shift, and kalman fifilter in time-lapse microscopy," *IEEE Transactions on Circuits and Systems I: Regular Papers*, vol. 53, no. 11, pp. 2405–2414, Nov 2006.

13. S. Ali and A. Madabhushi, "An integrated region-, boundary-, shapebased active contour for multiple object overlap resolution in histological imagery," *IEEE Transactions on Medical Imaging*, vol. 31, no. 7, pp. 1448–1460, July 2012.

14. M. Veta, P. J. van Diest, R. Kornegoor, A. Huisman, M. A. Viergever, and J. P. Pluim, "Automatic nuclei segmentation in h&e stained breast cancer histopathology images," *PloS one*, vol. 8, no. 7, p. e70221, 2013.

15. Y. Al-Kofahi, W. Lassoued, W. Lee, and B. Roysam, "Improved automatic detection and segmentation of cell nuclei in histopathology images," *IEEE Transactions on Biomedical Engineering*, vol. 57, no. 4, pp. 841–852, April 2010.

16. H. Kong, M. Gurcan, and K. Belkacem-Boussaid, "Partitioning histopathological images: An integrated framework for supervised colortexture segmentation and cell splitting," *IEEE Transactions on Medical Imaging*, vol. 30, no. 9, pp. 1661–1677, Sept 2011.

17. H. Chang, J. Han, A. Borowsky, L. Loss, J. W. Gray, P. T. Spellman, and B. Parvin, "Invariant delineation of nuclear architecture in glioblastoma multiforme for clinical and molecular association," *IEEE Transactions on Medical Imaging*, vol. 32, no. 4, pp. 670–682, April 2013.

18. M. Zhang, T. Wu, and K. M. Bennett, "Small blob identification in medical images using regional features from optimum scale," *IEEE Transactions on Biomedical Engineering*, vol. 62, no. 4, pp. 1051–1062, April 2015.

19. H. Irshad, A. Veillard, L. Roux, and D. Racoceanu, "Methods for nuclei detection, segmentation, and classification in digital histopathology: A review—current status and future potential," *IEEE Reviews in Biomedical Engineering*, vol. 7, pp. 97–114, 2014.

20. E. Moen, D. Bannon, T. Kudo, W. Graf, M. Covert, and D. Van Valen, "Deep learning for cellular image analysis," *Nature methods*, vol. 16, no. 12, pp. 1233–1246, 2019.

21. J. Long, E. Shelhamer, and T. Darrell, "Fully convolutional networks for semantic segmentation," in *Proceedings of the IEEE conference on computer vision and pattern recognition*, 2015, pp. 3431–3440.

22. N. Kumar, R. Verma, A. Arora, A. Kumar, S. Gupta, A. Sethi, and P. H. Gann, "Convolutional neural networks for prostate cancer recurrence prediction," in *Medical Imaging 2017: Digital Pathology*, vol. 10140. International Society for Optics and Photonics, 2017, p. 101400H.

23. O. Ronneberger, P. Fischer, and T. Brox, "U-net: Convolutional networks for biomedical image segmentation," in *International Conference on Medical image computing and computer-assisted intervention*. Springer, 2015, pp. 234–241.

24. Z. Zhou, M. M. R. Siddiquee, N. Tajbakhsh, and J. Liang, “Unet++: A nested u-net architecture for medical image segmentation,” in *Deep Learning in Medical Image Analysis and Multimodal Learning for Clinical Decision Support*. Springer, 2018, pp. 3–11.

Pravin R. Kharat

Research Scholar

Dr. Abhijeet V. Nandedkar

Supervisor